

Supporting Information

Schindler *et al.* 10.1073/pnas.0805108105

SI Materials and Methods

Fertilization was begun on June 26, 1969. During the 1st year of fertilization, the lake was fertilized during the ice-free season with weekly doses of Na_2HPO_4 and NaNO_3 , which were mixed and dissolved in the bottom of a boat. The boat was then driven around the lake while the nutrient mixture was allowed to pass into the lake through the boat's drain hole (1). From 1970 on, H_3PO_4 was substituted as a phosphorus source because of its higher solubility. The annual nutrient load each year was delivered in 17–20 weekly doses between May and October.

Beginning in 1975, after discovery of the rapid rate of horizontal mixing in the lake (2), weekly fertilizer doses were mixed in a 200-liter barrel and allowed to drip slowly into the lake. It usually required 4–5 days for the barrel to drain each time. More details are given in refs. 1 and 3–5.

When inputs of nutrients from precipitation, runoff, and estimated nitrogen fixation were added to the fertilizer inputs given in Table 1, the N:P ratio reaching the lake each year was 13.2–14.4 by weight in 1969–1974, 6.2–8.4 in 1975–1989, and 1.7–3.7 in 1990 and thereafter (5).

On each sampling date, temperature profiles were measured at 1-m intervals (0.25- to 0.5-m intervals through the thermocline). The epilimnion of the lake was sampled weekly at 0, 1, and 2 m during the ice-free season in 1969–1971 and every 2nd week thereafter. There was little evidence of stratification within the

epilimnion and an extremely strong summer thermocline between 2 and 3 m. After 1975, an integrated epilimnion sample was substituted for the 0- to 2-m samples in earlier years. Tests in 1975 showed that these were <11% different from calculations based on averages of discrete samples from the epilimnion. Samples were returned within 1 h to the Experimental Lake Area analytical laboratory, where they were filtered through Whatman GF/C glass fiber filters and analyzed the same day for nitrate, ammonium, total dissolved nitrogen, and total dissolved phosphorus. Particulate samples were frozen and returned to the Freshwater Institute for analysis. Chlorophyll *a* samples were frozen and analyzed fluorometrically. Details of analytical procedures are outlined in ref. 6. Secchi depths were also measured.

Phytoplankton were sampled from discrete water samples before 1975 and using integrated epilimnion and metalimnion samples after that. Methods are given in ref. 7. Samples for identification and enumerations were preserved in acid Lugol's solution and analyzed by using an inverted microscope (5). Heterocysts were counted when N-fixing cyanobacteria were present. These were found to be an excellent predictor of nitrogen fixation rates, based on calibrations with acetylene reduction in 1988–1992 (5). Here, N fixation in all years is estimated from heterocyst counts and the equation of ref. 5.

To facilitate plotting of 37 years of data, we have averaged epilimnion results for each ice-free season.

1. Schindler DW, Armstrong FAJ, Holmgren SK, Brunskill GJ (1971) Eutrophication of Lake 227, Experimental Lakes Area (ELA), northwestern Ontario, by addition of phosphate and nitrate. *J Fish Res Board Can* 28:1763–1782.
2. Quay PD, Broecker WS, Hesslein RH, Fee EJ, Schindler DW (1979) *Isotopes in Lake Studies* (International Atomic Energy Agency, Vienna), pp 175–194.
3. Schindler DW, *et al.* (1973) Eutrophication of Lake 227 by addition of phosphate and nitrate: The second, third, and fourth years of enrichment 1970, 1971, and 1972. *J Fish Res Board Can* 30:1415–1440.
4. Cruikshank DR (1984) *Whole-Lake Chemical Additions in the Experimental Lakes Area, 1969–1983* (Fish Mar Serv Data Rep 449) (Canadian Department of Fisheries and Oceans, Winnipeg, Canada).
5. Findley DL, Hecky RE, Hendzel LL, Stainton MP, Regehr GW (1994) Relationship between N_2 -fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. *Can J Fish Aquat Sci* 51:2254–2266.
6. Stainton MP, Capel MJ, Armstrong FAJ (1977) *The Chemical Analysis of Fresh water* (Fish Mar Serv Misc Spec Publ 25) (Canadian Department of Fisheries and Oceans, Winnipeg, Canada), 2nd Ed.
7. Shearer JA, DeBruyn ER, DeClercq DR, Schindler DW, Fee EJ (1985) Manual of phytoplankton primary production methodology. (Canadian Department of Fisheries and Oceans, Winnipeg, Canada). *Can Tech Rep Fish Aquat Sci* 1341.